

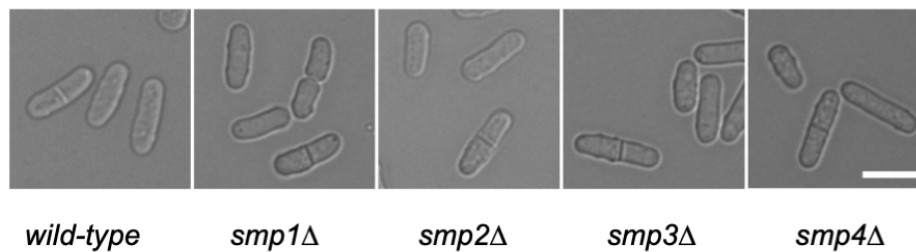
Additional file 3

Figure S1: Multiple sequence alignment of Smp1-Smp4 proteins from different *Schizosaccharomyces* species. Abbreviations: Sp, *S. pombe*; So, *S. octosporus*; Sj, *S. japonicus*; Sc, *S. cryophilus*. Homologues were identified by BLAST searching the UNIPROTKB_FUNGI database at the Universal Protein Resource (UniProt). Alignments were generated using Clustal Ω (1.2.4).

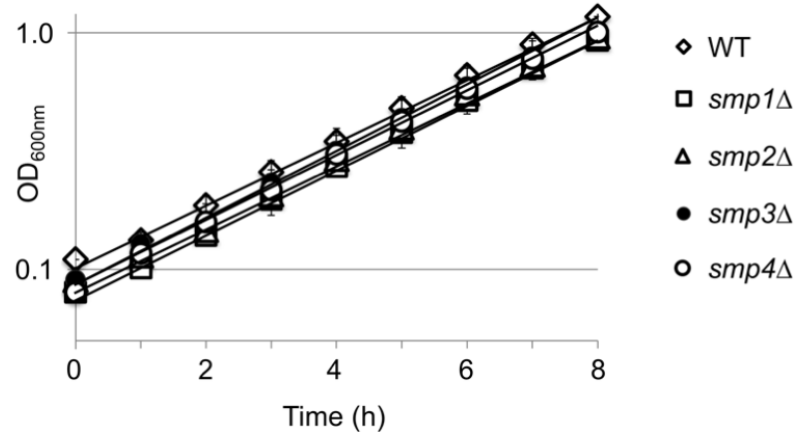
SpSmp1	MSPRASLEKELNSARLLHATINAMDVYTQNLINELQEARDSINDLQRAHERLKLVGAKAKLQIKRDEKKPKS	72
SjSmp1	MQQHRKQQLKEAKMLHATVNAVLDLYLSELLEDIRASSDAIKKLDTSNKFENTLSQA---IKKTGKNA--	65
	: : : : * : . * : : * : : * : * : * : . : * : : : : : : : * : * : . * : : : : : : : * : * : *	
SpSmp2	MQHNKENHFVEDAKQFQEKAKLYQGNYITLDGELITIIPSSKEGFRSCKSLYYKKKQPIPGR	62
ScSmp2	MEQDKENQLNLPKLSIPQDIKVFQGNYYITLDGEQTP-NPDGTTEFKTKALYYKSKKPIPGR	61
	* : : : * : * : : : : : : : * : : : * : : : * : : : * : : : * : : : * : : : * : : : *	
SpSmp3	MAQTFQEKQQSRRIKMSTGNFFSRMWNNAVVFVGFGAAIGASVANAALGACCG-	51
SjSmp3	MATFQEKQQRRIKMRTGNFFSRMWNNAVIFGFGAAIGATVANAALGACCG-	50
ScSmp3	MGRTFQEKQQSRRVKMSTGNIFSRVWNSVVFVGFGAALGATAASAMIGACCG-	51
SoSmp3	MARTFQEKQQGRRMKMSTGNIFSRVWNSVVFVGFGAALGATAASAMLGACCGG	52
	***** * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
SpSmp4	MSAPYKNLDRDTKHHPKLNETERNLNRGWGDVKKEELYEDLAQSDADKQLAEDKMETKYEKSPPAPSD	69
SoSmp4	MSPAPYKDLKSDTVHTHPPIHESERREEHGWGSLKKEPLMEDQAEQDAKADLSKDKKEVKEEASRPPPSV	70
ScSmp4	MSPAPYKDLKSDTVHTHPAIRESERREHGWGDLNKEPLMEDQAEERDAKNDLSKDQKEVKEQASRPAPNN	70
SjSmp4	MPYMPQVKHGEVIRDDRHSHTGLKISDRADTRGWGDVKHEREFEELAERDAETDQRRDTEEVQKEPELLPPQ-	72
	. : : : * : * : . : * : : * : : * : : * : * : . : * : * : : . * : * : : . *	

Figure S2: Phenotypic analysis of *smp1* Δ - *smp4* Δ cells. **A.** Microscopic images of wild-type, *smp1* Δ , *smp2* Δ , *smp3* Δ and *smp4* Δ cells growing exponentially in YE4S medium at 32°C. Scale bar: 10 μ m. **B.** Growth rates (OD_{600nm} versus time) of wild-type (WT), *smp1* Δ , *smp2* Δ , *smp3* Δ and *smp4* Δ cells growing in YE4S medium at 32°C. **C.** Examples of meiotic asci formed by self-crossing wild-type, *smp1* Δ , *smp2* Δ , *smp3* Δ and *smp4* Δ strains of opposite mating type on SPA agar at 25°C for 48 hours. The figure was assembled from multiple representative images of the same crosses. Scale bar: 10 μ m.

A



B



C

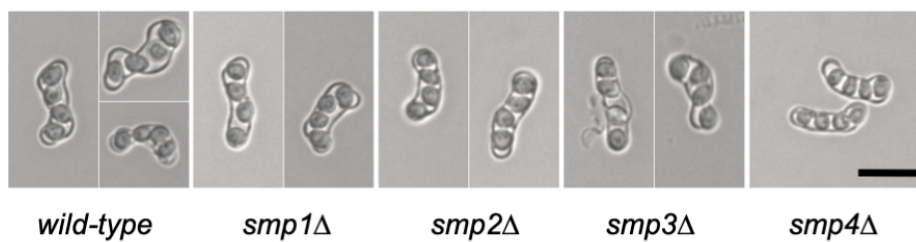


Figure S3: Insertion sites relative to sgRNA and PAM sequences. Sequences of *smp1-S1* – *smp1-S3* and *smp1-S1* – *smp3-S3* alleles are shown. PAM sequences are shown in bold, sequence corresponding to sgRNA in red. Note that the insertion in *smp1-S3* also deletes two nucleotides. See Table S6 for identity of inserted *Oncorhynchus* sequences.

	sgRNA	
<i>smp1</i>	ACGATTTACAGAGTAAGTAA CGG GTACATTGAAGATTGC	
<i>smp1-S1</i>	ACGATTTACAGAGTAAGT---384---AA CGG GTACATTGAAGATTGC	
<i>smp1-S2</i>	ACGATTTACAGAGTAAGT---183---AA CGG GTACATTGAAGATTGC	
<i>smp1-S3</i>	ACGATTTACAGAGTAA-----73---AA CGG GTACATTGAAGATTGC	
	inserted sequences (bp)	PAM

	sgRNA	
<i>smp3</i>	CGCACCGAAGCCGAACACGA CGG CATTCCACATTCTAGA	
<i>smp3-S1</i>	CGCACCGAAGCCGAAC---121---ACGA CGG CATTCCACATTCTAGA	
<i>smp3-S2</i>	CGCACCGAAGCCGAAC---121---ACGA CGG CATTCCACATTCTAGA	
<i>smp3-S3</i>	CGCACCGAAGCCGAAC---113---ACGA CGG CATTCCACATTCTAGA	
	inserted sequences (bp)	PAM